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Glucose and cationic amino acid transporter expression in growing chickens (*Gallus gallus domesticus*)

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Abstract

Tissue glucose transporter (GLUT1–3) and cationic amino acid transporter (CAT1–3) mRNA expression was determined in growing broiler chicks posthatch. In two experiments, tissues were either collected on days 1, 3 and 7 or days 1 and 14 posthatch. Heart and liver were the only tissues expressing a GLUT isoform on day 1. All tissues expressed a GLUT isoform on day 7 except for the thymus. Most tissues expressing a CAT isoform on day 1 decreased mRNA levels through day 7 ($P<0.05$), except for bursa CAT-1 which tended to increase ($P=0.05$). The thymus and spleen did not express any CAT isoform mRNA until day 7. The liver was the only tissue expressing GLUT-2 mRNA through day 14. On day 14, GLUT-1, CAT-1 and CAT-2 mRNA were differentially expressed across tissues ($P<0.05$). High-affinity GLUT and CAT mRNA expression was highest in the heart and bursa, respectively ($P<0.05$). Total CAT mRNA expression was greatest in the bursa ($P<0.05$). The thymus had the lowest high affinity GLUT and total CAT mRNA expression on day 14 posthatch. Therefore, T lymphocytes within the thymus may be most susceptible to glucose and cationic amino acid supply.

Keywords: Arginine; Glucose; Growth; Lymphocyte; Lysine; Ontogeny; Transport

1. Introduction

Membrane transporters are the cellular gateway for the flux of ions and molecules. Membrane transporters maintain osmotic stability, control ion concentrations and permit uptake and release of macromolecules, nutrients and wastes or toxins (Yan, 2003). Whole genome analysis in lower organisms indicates that 10% of the genes encode transport proteins (Paulsen et al., 1998), and functional and phylogenetic classification of transport proteins has identified nearly 400 transport system families (Busch and Saier, 2002). The two primary transporters are channels and carriers, and carriers represent a much larger and varied group of proteins responsible for nutrient uptake (Saier, 1999). Membrane transporters for lipids, amino acids, glucose, vitamins, minerals and products of intermediary metabolism are important in nutrient acquisition, possession and metabolism.

In mammals, glucose transporters (GLUTs) are organized into three facilitative transporter classes based upon multiple sequence alignment (Joost et al., 2002). GLUT1–4 comprise Class I facilitative glucose transporters and have received the most comprehensive study to date (Mueckler, 1994). Class II and III isoforms transport fructose and glucose, respectively, and include novel GLUT isoforms whose regulation and functional properties are being identified (Wood and Trayhurn, 2003). Class I facilitative transporters are widely distributed across tissues and have a specific role in the control of whole-body glucose homeostasis (Uldry and Thorens, in press). GLUT-1, GLUT-3 and GLUT-4 are high-affinity isoforms (K_m=2–5 mM) and GLUT-2 is a low-affinity isoform (K_m=17 mM) (Mueckler, 1994).

Abbreviations: CAT, cationic amino acid transporter; Gastroc, gastrocnemius; GLUT, glucose transporter; LOW, expressed below the level needed for accurate quantification; nd, not detectable; Pec, pectoralis.

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Amino acids are transported across the plasma membrane by independent systems that often overlap in their substrate specificities (Palacin et al., 1998). Cationic amino acids are transported by three gene families: the system y^+ cationic amino acid transport (CAT) family; the glycoprotein-associated amino acid transporter (gpaAT) family, also known as the light chains of the heterodimeric amino acid transporters (HAT); and system $B^{0,+}$ of the (Na^+/Cl^-)-dependent transporter family (Deves and Boyd, 1998). HATs have the greatest number and variety of amino acid transport systems identified to date in mammals and absorb and reabsorb amino acids from the intestinal lumen and glomerular filtrate, respectively (Chillaron et al., 2001). In contrast to HATs, CATs and system $B^{0,+}$ accumulate substrate within a cell by transporting lysine, arginine and ornithine against their concentration gradient (Stein, 1990). The four mammalian CAT isoforms, CAT-1, CAT-2A, CAT-2B and CAT-3, differ in tissue expression and transport kinetics (Deves and Boyd, 1998; MacLeod and Kakuda, 1996). CAT-1, CAT-2B and CAT-3 mediate high-affinity transport (K_m=70–400 μM), while CAT-2A mediates low-affinity transport (K_m=2–5 mM). In mammals, CAT tissue expression is widespread and system $B^{0,+}$ tissue expression is limited to specialized tissues, such as the lung and salivary gland (Sloan and Mager, 1999). Therefore, CATs are the primary amino acid transport system used by tissues to concentrate lysine, arginine and ornithine into cellular amino acid pools for use in nitrogen metabolism (Broer, 2002).

The ability of tissues to adapt to changes in dietary composition or nutrient availability is reflected in the changes in the types and amounts of nutrient transporters. The hatchling chick is adapted to metabolize high lipid and protein substrate from utilization of egg yolk and albumen during embryogenesis. Despite these adaptations, chicks are fed seed-based diets composed of high glucose and relatively low protein. Therefore, the rapid shift in dietary composition that occurs following hatch makes the hatchling chick a useful model to examine mechanisms of metabolic adaptation. Several functions are highly compartmentalized in chickens, enhancing the interpretation of tissue specific data. For example, lymphocyte development is highly compartmentalized with T-cell development in the thymus and B-cell development in the bursa. Therefore, the purpose of these experiments was to determine the ontogeny and tissue differences in GLUT and CAT mRNA expression in growing broiler chickens during the first 2 weeks posthatch.

2. Materials and methods

2.1. Animals

Male Cobb broiler hatchlings (Foster Farms, Delhi, CA) were provided ad libitum access to water and a corn–soybean meal diet (Table 1) prepared according to the National Research Council recommendations for a young growing broiler chick (NRC, 1994). Hatchlings were selected for uniform body mass from a threefold larger population and were raised in Petersime brooder batteries (Petersime Incubator, Gettysburg, OH, USA) located in an environmentally controlled room (25 °C; 18 h light:6 h darkness). All experiments and procedures were approved by the UC Davis Campus Animal Care and Use Committee.

Table 1
Composition of diets fed to chicks

	g/kg
Ingredient	
Corn	501.8
Soy meal (48% protein)	394.1
Meat with bone meal	30.0
Poultry fat	40.8
Calcium carbonate	10.5
Defluorinated phosphate	11.1
Sodium chloride	3.4
DL methionine	2.4
Vitamin and mineral premix[1]	2.4
Bacitracin MD	0.5
Salinomycin	0.5
Roxarsone 20%	0.2
Mold inhibitor	2.5
Calculated composition	
ME (MJ/kg)	12.85
Crude protein (%)	24.11
Crude fat (%)	6.74
Crude fiber (%)	2.71
Available Lys (%)	1.32
Available Met+Cys (%)	0.94

[1] Vitamins and minerals were provided in the form and level described in the NRC Standard Reference Diet for Chicks (NRC, 1994).

2.2. Experimental design

In experiment one, tissue GLUT and CAT mRNA expression was determined on days 1, 3 and 7 posthatch. On the day of hatch (day 0), four pens of three hatchlings per pen were randomly assigned to each of the three time points. In experiment two, tissue GLUT and CAT mRNA expression was determined on days 1 and 14 posthatch. On the day of hatch (day 0), six pens of three hatchlings per pen were randomly assigned to each of the two time points.

2.3. Tissues sampling

On the day of sampling, organs were collected from each chick for GLUT and CAT mRNA analysis. Chicks were killed by CO_2 overdose and tissues were immediately excised from each chick. The spleen, bursa, heart, left liver lobe, gastrocnemius (gastroc), at least three thymus lobes and a pectoralis (pec) sample lateral to the keel bone were collected. All tissue samples were immediately frozen between two aluminum plates in liquid N and stored at −80 °C until further analysis.

2.4. RNA isolation and RT-PCR

Total RNA was isolated using the RNAgents Total RNA Isolation System (Promega, Madison, WI, USA). Tissue samples (25 mg) of similar weight (±3 mg) were homogenized with a Polytron grinder (Brinkman Instruments, Westbury, NY, USA) and total RNA was isolated according to the manufacturer's instructions. Optical density at 260 nm was used to determine RNA concentrations. Equal amounts of RNA from chicks of the same pen were pooled in both experiments. RNA samples were reverse transcribed in a Programmable Thermal Controller (PTC)-100 (MJ Research, Watertown, MA, USA). Two micrograms of RNA was converted to cDNA in a 20 μl reaction volume containing 1× reaction buffer (50 mM Tris–HCl [pH 8.3], 75 mM KCl, 3 mM $MgCl_2$, 10 mM DTT), 10 mM DTT, 0.5 mM dNTPs, 0.5 μg of oligo$(dT)_{15}$ primer, 8 units of RNAsin and 100 units of M-MLV reverse transcriptase (all from Promega) at 40 °C for 1 h, and then 95 °C for 10 min.

Quantitative real-time PCR analysis of GLUT-1, GLUT-2, GLUT-3, CAT-1, CAT-2, CAT-3 and β-actin mRNA was performed with a Roche LightCycler (Roche Diagnostics, Indianapolis, IN, USA). Primers to chicken GLUT-1, GLUT-2 and GLUT-3 were designed in the open reading frame using published chicken sequences (Table 2; Wang et al., 1994; White et al., 1994; Wagstaff et al., 1995). Chicken CAT-1, CAT-2 and CAT-3 full-length gene sequences have not been cloned; therefore, primers were designed from chicken expressed sequence tags (ESTs) obtained from the BBSRC Chicken EST Database (Table 2). All EST sequences had >80% nucleotide homology (Standard nucleotide–nucleotide BLAST, NCBI) and >76% amino acid homology (Nucleotide query-Protein database BLASTX, NCBI) compared to their cloned mammalian counterparts. ClustalW multiple sequence alignment of each chicken CAT EST with the cloned mammalian CAT sequence occurred in the open reading frame of all respective mammalian CATs. Mammalian CAT-2 contains two splice variants (Closs et al., 1993), and chicken CAT-2 EST homology to mammalian CAT-2 occurred in the shared region of the mammalian CAT-2 splice variants. Preliminary experiments confirmed all primer pairs spanned an intron. PCR products from each gene were visualized by gel electrophoresis on 1% agarose stained with ethidium bromide to ensure a single product was produced at the predicted size. Single band PCR products for each gene were excised and cDNA was purified with the QIAquick Gel Extraction Kit (Qiagen, Valencia, CA, USA) for sequencing (Davis Sequencing, Davis, CA, USA). All PCR products had >99% homology to their respective gene or EST sequence (Standard nucleotide–nucleotide BLAST, NCBI).

PCR reagents and reaction conditions were optimized for each gene utilizing the LightCycler DNA Master SYBR Green 1 kit (Roche Diagnostics). The 20 μl final reaction

Table 2
Primer sequences and PCR conditions for chicken GLUT-1, GLUT-2, GLUT-3, CAT-1, CAT-2, CAT-3 and β-actin mRNA[1]

Gene[2]	Primer sequence	Primer mix (μM)	$MgCl_2$ (mM)	PCR cycle conditions[3]	PCR product (bp)
GLUT-1	5′-GGCTTTGTCCTTTGAGATGC 3′-CGCTTTGTTCTCCTCATTGC	1	5	95 °C/1 s 58 °C/5 s 72 °C/17 s	328
GLUT-2	5′-TGTTCAGCTCCTCCAAGTACC 3′-ACAACGAACACATACGGTCC	2	5	95 °C/1 s 56 °C/5 s 72 °C/20 s	523
GLUT-3	5′-TTAGTTGGTTTGGGTGGCAT 3′-TCAGCATAGGGGAAGAGCAT	2	4	95 °C/1 s 58 °C/5 s 72 °C/12 s	260
CAT-1	5′-ATGTAGGTTGGGATGGAGCC 3′-AACGAGTAAGCCAGGAGGGT	2	4	95 °C/1 s 60 °C/4 s 72 °C/7 s	280
CAT-2	5′-GTTTCCTTCCTCATTGCTGC 3′-CCACTCCAGGCTCTTGCTAC	2	5	95 °C/1 s 62 °C/4 s 72 °C/7 s	200
CAT-3	5′-CAAGACTGGCTCTGCCTACC 3′-GGATCAACGCAAAGAAGTCC	2	5	95 °C/1 s 61 °C/3 s 72 °C/9 s	236
β-actin	5′-CTGACACCACACTTTCTACAATG 3′-GATCTTCATGAGGTAGTCCGTCAG	2	5	95 °C/1 s 63 °C/5 s 72 °C/16 s	350

[1] Abbreviations: base pairs (bp), cationic amino acid transporter (CAT), glucose transporter (GLUT), second (s).

[2] Primers developed for GLUT-1, GLUT-2, GLUT-3 and β-actin were based upon published sequences with the following respective GenBank accession numbers: L07300, Z22932, M37785 and L08165. Primer development for chicken CAT-1, CAT-2 and CAT-3 was based upon expressed sequence tags (EST) from the BBSRC Chicken EST Project with the following respective EST identification numbers: 603957065F1, 604130341F1 and 603508168F1.

[3] All PCR runs were performed with the Roche LightCycler and used an initial denaturation step at 95 °C for 120 s. PCR conditions for each primer pair consisted of 40 cycles followed by melting curve analysis by the LightCycler. The melting profile was obtained by increasing the temperature 20 °C/s from 65 to 95 °C while monitoring fluorescence continuously.

volume contained 2 μl reverse transcription product and 2 μl 10× LightCycler DNA Master SYBR Green 1 along with appropriate concentrations of primer and $MgCl_2$ (Table 2). PCR cycle conditions of all primer pairs used an initial 120-s denaturation step at 95 °C followed by 40 cycles of denaturing, annealing and extension. The melting profile of each sample was analyzed after every PCR run to confirm PCR product specificity. The melting profile was determined by heating samples at 65 °C for 30 s and then increasing the temperature at a linear rate of 20 °C/s to 95 °C while continuously monitoring fluorescence. Low amplicon number results in increased primer dimer formation, and prevents accurate quantification due to primer dimer nonspecific fluorescence (Vandesompele et al., 2002). Therefore, these samples were not quantified nor included in the analysis since expression levels were below the limit of accurate quantification. These samples are indicated as "LOW" in the results.

Quantification of mRNA by real-time PCR utilizes fluorescent chemistry to determine the threshold cycle (C_t) when the PCR product and fluorescence rises exponentially above background. C_t values for all samples were subtracted from the total cycle number (40) for each gene since C_t values are inversely related to the amount of PCR product. Differences in C_t values between samples can be quantified with the delta–delta equation, which describes the change in gene expression relative to a reference sample (Livak and Schmittgen, 2001). The delta–delta equation assumes the PCR amplification efficiency to be maximum and equal for all samples; however, actual PCR efficiencies vary over a wide range (Liu and Saint, 2002). Therefore, the PCR amplification efficiency for each sample was determined and used in the delta–delta equation. Sample PCR amplification efficiencies were determined in the log-linear phase with the LinRegPCR program (Ramakers et al., 2003). Additionally, the delta–delta equation subtracts sample and reference C_t values from an endogenous control; however, the endogenous control (β-actin) C_t was affected by tissue type and age in our studies ($P<0.05$), and therefore was removed from the equation. Instead, all data were normalized to a reference level and reported as the fold change from the reference. Fold change from the reference was calculated as $E_S^{(\text{Ct Sample})}/E_R^{(\text{Ct Reference})}$, where E_S and E_R are the sample and reference PCR amplification efficiencies, respectively. Different references were used in order to make comparisons of interest. In order to examine ontogeny, the value from the first day that an isoform was measurable served as the reference. In order to determine tissue isoform expression, tissue β-actin served as the reference. In order to compare between tissues, gastrocnemius served as the reference.

2.5. Statistical analysis

Dependent variables were analyzed by general linear model (JMP Software, SAS, Cary, NC, USA) using an analysis of variance (ANOVA). Prior to analysis, data were transformed to logarithms due to nonhomogeneity of variance (assessed with Levene's test). When main effects were significant ($P<0.05$), means comparisons were performed using Tukey's means comparisons. Data are reported as nontransformed means and pooled standard errors.

3. Results

3.1. Change in transporter mRNA expression from day 1 through day 7 and between day 1 and day 14

The changes in GLUT and CAT isoform mRNA expression in tissues from day 1 through day 7 are shown in Figs. 1 and 2, respectively. Data for each isoform within a

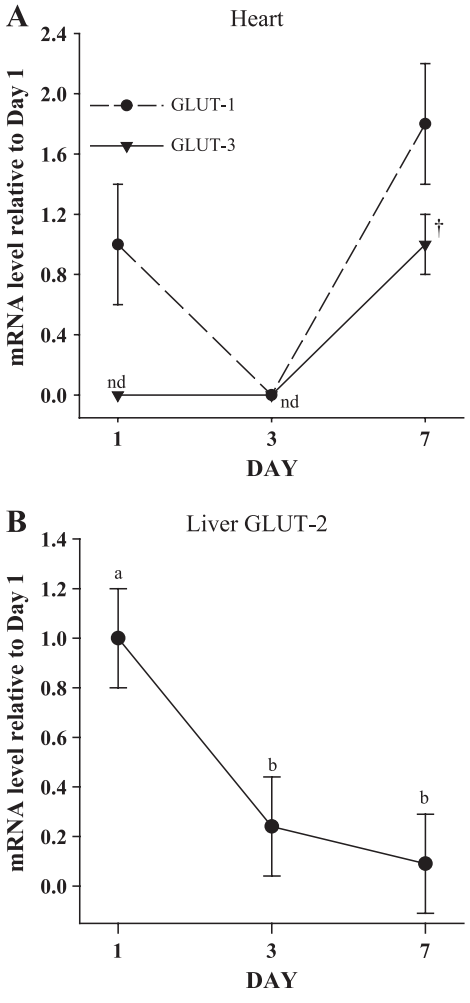

Fig. 1. Ontogeny of GLUT isoform mRNA in chicks from day 1 through day 7 posthatch for (A) heart and (B) liver. The level of mRNA for each isoform is expressed relative to the amount of that isoform on day 1. Values are means±S.E.M. ($n=4$). Means within a tissue not sharing a common superscript are significantly different ($P<0.05$). †Indicates measurable isoform mRNA on day 7 only. Abbreviations: glucose transporter (GLUT), not detectable (nd).

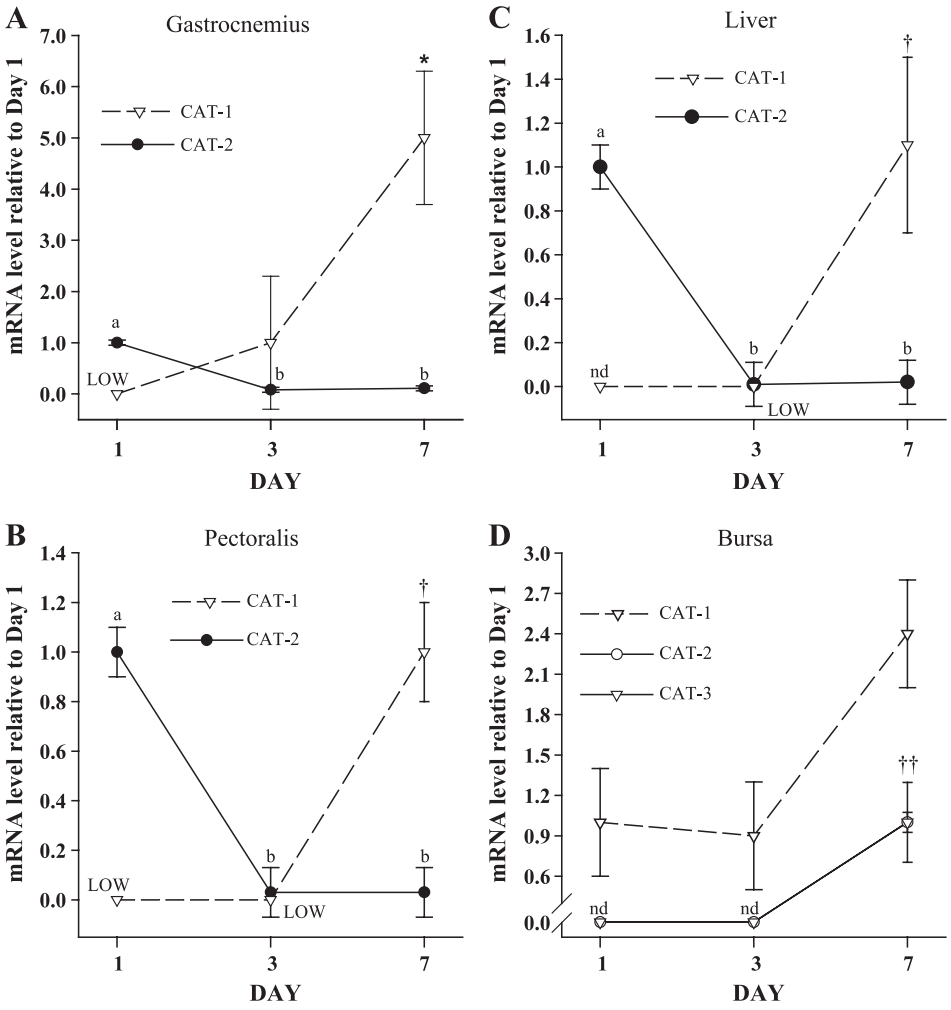

Fig. 2. Ontogeny of CAT isoform mRNA in chicks from day 1 through day 7 posthatch for (A) gastrocnemius, (B) pectoralis, (C) liver and (D) bursa. The level of mRNA for each isoform is expressed relative to the amount of that isoform on day 1. Values are means±S.E.M. (n=4). S.E.M. values were calculated only for isoforms with measurable expression. Means within a tissue not sharing a common superscript are significantly different (P<0.05). * Indicates significant difference within an isoform between day 3 and 7. † Indicates measurable isoform mRNA on day 7 only. Abbreviations: cationic amino acid transporter (CAT), expressed below the level needed for accurate quantification (LOW), not detectable (nd).

tissue were calculated by utilizing the isoform level on the first day that it was measurable as the reference, and is reported as the fold change from initial expression. GLUT-1 mRNA expression was undetectable on day 1 in all tissues except for the heart. Heart GLUT-1 mRNA levels were undetectable on day 3 and were similar to day 1 levels on day 7 (Fig. 1A, P>0.05). Gastrocnemius, pectoralis, bursa and spleen had levels of GLUT-1 mRNA that were too low

Table 3
Relative amount of each GLUT and CAT isoform mRNA within each individual tissue on day 7 posthatch[1,2]

Tissue	GLUT-1 ($\times10^{-3}$)	GLUT-2 ($\times10^{-3}$)	GLUT-3 ($\times10^{-3}$)	CAT-1 ($\times10^{-3}$)	CAT-2 ($\times10^{-3}$)	CAT-3 ($\times10^{-3}$)	S.E.M.	P-value
Heart	4.0^{b}	nd	19^{a}	LOW	0.8^{c}	nd	1.9	<0.01
Gastroc	0.6^{c}	nd	LOW	4.4^{b}	23^{a}	nd	1.7	<0.01
Pec	0.3^{c}	nd	LOW	1.4^{b}	24^{a}	LOW	1.4	<0.01
Liver	LOW	1.0^{b}	LOW	13^{a}	29^{a}	LOW	3.0	<0.01
Spleen	0.1^{b}	nd	2.2^{a}	LOW	0.03^{b}	0.04^{b}	0.3	<0.01
Bursa	3.0^{b}	nd	LOW	10^{a}	0.6^{c}	1.3^{bc}	0.7	<0.01
Thymus	LOW	nd	LOW	LOW	0.10	0.31	0.1	0.30

[1] Values are means±S.E.M. (n=4) of mRNA expression for each isoform relative to tissue β-actin expression. S.E.M. values were calculated only for isoforms with measurable expression. Means within a row not sharing a common superscript are significantly different (P<0.05).

[2] Abbreviations: cationic amino acid transporter (CAT), gastrocnemius (Gastroc), glucose transporter (GLUT), expressed below the level needed for accurate quantification (LOW), not detectable (nd), pectoralis (Pec).

Table 4
Relative amount of each GLUT and CAT isoform mRNA within each individual tissue on day 14 posthatch[1,2]

Tissue	GLUT-1 ($\times 10^{-3}$)	GLUT-2 ($\times 10^{-3}$)	GLUT-3 ($\times 10^{-3}$)	CAT-1 ($\times 10^{-3}$)	CAT-2 ($\times 10^{-3}$)	CAT-3 ($\times 10^{-3}$)	S.E.M.	*P*-value
Heart	2.0^{b}	nd	32^{a}	nd	1.4^{b}	1.2^{b}	3.0	<0.01
Gastroc	1.5^{c}	nd	97^{a}	8.7^{d}	11^{b}	1.8^{c}	8.0	<0.01
Pec	1.3^{c}	nd	142^{a}	LOW	16^{b}	1.4^{c}	16	<0.01
Liver	1.0^{e}	516^{a}	81^{ab}	9.5^{cd}	18^{bc}	2.5^{de}	160	<0.01
Spleen	0.2^{b}	nd	15^{a}	LOW	0.05^{c}	0.2^{b}	2.0	<0.01
Bursa	0.2^{bc}	nd	16^{a}	3.1^{a}	0.08^{c}	0.3^{b}	1.5	<0.01
Thymus	0.2^{b}	nd	33^{a}	LOW	0.3^{b}	0.8^{b}	5.0	<0.01

[1] Values are means±S.E.M. (n=4) of mRNA expression for each isoform relative to tissue β-actin expression. S.E.M. values were calculated only for isoforms with measurable expression. Means within a row not sharing a common superscript are significantly different (P<0.05).

[2] Abbreviations: cationic amino acid transporter (CAT), gastrocnemius (Gastroc), glucose transporter (GLUT), expressed below the level needed for accurate quantification (LOW), not detectable (nd), pectoralis (Pec).

to quantify (LOW) on day 3 and had measurable levels on day 7. Liver and thymus GLUT-1 mRNA levels were LOW through day 7. GLUT-2 mRNA expression was undetectable through day 7 in all tissues except for the liver. Liver GLUT-2 mRNA expression decreased 11-fold during the first week posthatch (Fig. 1B, P<0.05). GLUT-3 mRNA expression was not detectable (nd) in any tissue on day 1 posthatch. Heart and spleen were the only tissues expressing GLUT-3 mRNA on day 7 posthatch. GLUT-3 mRNA expression was LOW in liver, thymus, gastrocnemius, pectoralis and bursa on day 7 posthatch.

Unlike the GLUT isoforms, most tissues had measurable levels of at least one CAT isoform on day 1 posthatch. CAT-2 mRNA levels in gastrocnemius, liver and pectoralis decreased during the first week posthatch (Fig. 2, P<0.05). CAT-1 mRNA levels in gastrocnemius, liver and pectoralis increased during the first week posthatch (P<0.05). Gastrocnemius and liver CAT-3 mRNA expression never reached measurable levels, and pectoralis CAT-3 mRNA expression was LOW. Bursa CAT-1 mRNA expression tended to increase from the day 1 level on day 7 (Fig. 2D, P=0.054). Bursa CAT-2 and CAT-3 mRNA levels were measurable on day 7 only. Heart CAT-1 mRNA expression never reached measurable levels. Heart CAT-2 and CAT-3 mRNA expression were detectable only on days 7 and 1, respectively. Spleen and thymus CAT isoforms were undetectable on day 1, and CAT-2 and CAT-3 mRNA were measurable in both tissues on day 7.

Because several tissues did not express some transporter isoforms during the first week posthatch in the first experiments, a second experiment determined GLUT and CAT isoform mRNA expression on day 1 and day 14. Tissue expression of GLUT and CAT isoforms on day 1 was identical between experiments. GLUT-1 mRNA levels on day 14 increased in heart (19-fold) and GLUT-2 mRNA levels decreased in liver (3.2-fold) from day 1 levels (P<0.05). CAT-3 mRNA levels on day 14 increased in heart (30-fold) from day 1 levels (P<0.05). CAT-2 mRNA levels in gastrocnemius (44-fold), pectoralis (794-fold) and liver (14-fold) increased from day 1 levels (P<0.05). CAT-1 mRNA levels tended to increase in the bursa from day 1 levels (P=0.09).

3.2. Relative amount of transporter isoform mRNA in each tissue on day 7 and day 14

In order to compare the relative amount of each GLUT and CAT isoform mRNA within a single tissue, PCR values for each isoform were expressed relative to that for β-actin. β-actin mRNA levels differed across tissues and days (P<0.05), so the β-actin mRNA levels for each tissue on each day served as the reference. On day 7, GLUT-3 was the predominant isoform expressed in the heart, which was almost fivefold higher than GLUT-1 and 20-fold higher than CAT-2 (Table 3). The gastrocnemius and pectoralis had similar expression patterns, with high levels of CAT-2 mRNA and LOW or nondetectable levels of GLUT-2, GLUT-3 and CAT-3 mRNA. The liver had high levels of CAT-1 and CAT-2 mRNA and was the only tissue expressing GLUT-2 mRNA. GLUT-3 was the predominant isoform in the spleen, which was 22-fold higher than GLUT-1 (P<0.05). CAT-1 was the predominant isoform expressed in the bursa, which was 16-fold and 7.5-fold higher than CAT-2 and CAT-3, respectively (P<0.05). Thymus GLUT-1, GLUT-2, GLUT-3 and CAT-1 mRNA levels were LOW

Table 5
Relative level of GLUT and CAT isoform mRNA expression across tissues on day 14 posthatch[1,2,3]

Tissue	GLUT-1	GLUT-3	CAT-1	CAT-2	CAT-3
Heart	6.1^{a}	1.6	nd	0.6^{b}	4.7
Gastroc	1.0^{b}	1.1	1.2^{b}	1.1^{a}	1.5
Pec	1.3^{ab}	2.2	LOW	2.1^{a}	1.8
Liver	0.9^{b}	1.2	3.2^{ab}	2.3^{a}	2.8
Spleen	2.1^{ab}	2.2	LOW	0.07^{c}	2.6
Bursa	1.5^{ab}	1.7	7.7^{a}	0.09^{bc}	3.1
Thymus	0.8^{b}	1.6	LOW	0.12^{bc}	2.4
S.E.M.	0.6	0.4	1.6	0.2	1.2
P-value	<0.01	0.18	<0.01	<0.01	0.28

[1] Values are means±S.E.M. (n=6) of mRNA expression for each isoform relative to gastrocnemius. Means within a column not sharing a common superscript are significantly different (P<0.05).

[2] GLUT-2 was not analyzed since this isoform was expressed in liver only.

[3] Abbreviations: cationic amino acid transporter (CAT), gastrocnemius (Gastroc), glucose transporter (GLUT), expressed below the level needed for accurate quantification (LOW), not detectable (nd), pectoralis (Pec).

or not detectable. Thymus CAT-2 and CAT-3 mRNA levels did not differ ($P>0.05$).

The expression pattern in the heart on day 14 was similar to that observed on day 7 (Table 4). On day 14, GLUT-3 became the predominant isoform expressed in the pectoralis and gastrocnemius. As on day 7, the liver was the only tissue expressing GLUT-2 mRNA on day 14, and GLUT-2 was the predominant liver isoform. On day 14, GLUT-3 became the predominant isoform expressed in the spleen and thymus ($P<0.05$). GLUT-3 and CAT-1 were the predominant isoforms in the bursa. Bursa GLUT-3 mRNA levels were 32-fold higher than GLUT-1 mRNA levels, and CAT-1 mRNA levels were 39-fold and 10-fold higher than CAT-2 and CAT-3 mRNA levels, respectively ($P<0.05$).

3.3. Relative level of transporter isoform expressed across tissues on day 14

In order to compare the amount of a transporter isoform between the different tissues, PCR values were normalized for the amount expressed in gastrocnemius (Table 5). GLUT-1 mRNA expression in the heart was 7.6-fold greater than the thymus ($P<0.05$), while GLUT-3 mRNA expression was similar across tissues ($P>0.05$). GLUT-2 mRNA was expressed in the liver only. CAT-1 mRNA expression in the bursa was 6.4-fold greater than the gastrocnemius, while CAT-2 mRNA expression in the liver was 33-fold greater than the spleen ($P<0.05$). PCR values were not normalized to gastrocnemius on days 1, 3 or 7 due to the limited GLUT and CAT isoform mRNA expression at these ages.

In order to compare functional categories of transporters, the high-affinity GLUT isoforms (GLUT-1 and GLUT-3), high-affinity CAT isoforms (CAT-1 and CAT-3), and total CAT isoforms (CAT-1, CAT-2 and CAT-3) were summed (Table 6). Values were normalized to the respective amounts expressed in gastrocnemius. Total high affinity GLUT mRNA expression in heart was 3.5-fold greater than the liver on day 14 ($P<0.05$). Day 14 total CAT mRNA expression in the bursa was 3.8-fold greater than the thymus ($P<0.05$), while total high affinity CAT mRNA expression in the bursa was 4.5-fold greater than the pectoralis ($P<0.05$).

Table 6
Relative level of high affinity GLUTs and total and high affinity CATs expressed in tissues on day 14 posthatch[1,2,3]

Tissue	High-affinity GLUT[4]	Total CAT[5]	High-affinity CAT[6]
Heart	3.9[a]	2.0[ab]	2.2[ab]
Gastroc	1.0[b]	1.3[ab]	1.1[ab]
Pec	1.7[ab]	1.3[ab]	0.8[b]
Liver	1.1[b]	2.8[ab]	2.5[ab]
Spleen	2.2[ab]	1.0[ab]	1.2[ab]
Bursa	1.7[ab]	3.0[a]	3.6[a]
Thymus	1.2[b]	0.8[b]	1.0[ab]
S.E.M.	0.4	0.5	0.6
P-value	<0.01	0.02	0.01

[1] Values are means±S.E.M. ($n=6$) of mRNA expression relative to gastrocnemius. Means within a column not sharing a common superscript are significantly different ($P<0.05$).

[2] Total GLUT was not included since GLUT-2 was expressed in the liver only.

[3] Abbreviations: cationic amino acid transporter (CAT), gastrocnemius (Gastroc), glucose transporter (GLUT), pectoralis (Pec).

[4] Sum of GLUT-1 and GLUT-3 mRNA expression within a tissue normalized to the summed level in gastrocnemius.

[5] Sum of CAT-1, CAT-2 and CAT-3 mRNA expression within a tissue normalized to the summed level in gastrocnemius.

[6] Sum of CAT-1 and CAT-3 mRNA expression within a tissue normalized to the summed level in gastrocnemius.

4. Discussion

The purpose of these experiments was to determine the changes in the type and amount of tissue GLUT and CAT isoform mRNA expression in growing chicks posthatch. Characterization and ontogeny of GLUT expression in tissues has been described in mammals (Mueckler, 1994); however, characterization of chicken GLUT expression in tissues is limited to liver (Wang et al., 1994), embryonic tissue (Carver et al., 2001) and embryo fibroblasts (White and Weber, 1988; White et al., 1994; Wagstaff et al., 1995). Unlike mammals, identification of insulin-responsive GLUT-4 of chickens has remained elusive, and still has not been definitively characterized at either the gene or protein level (Carver et al., 2001). Characterization of tissue CAT isoform expression has also been extensively reported in humans, rats, mice and a variety of cell lines (Deves and Boyd, 1998). However, the ontogeny of CAT expression has not been determined in any species. In the present study, patterns of mRNA expression for GLUT and CAT isoform in growing chicks were age and tissue dependent and may indicate differences in tissue priority and transport capacity for glucose and cationic amino acids.

4.1. Ontogeny of transporter mRNA expression

The changes in GLUT and CAT mRNA expression during the first week posthatch indicate a transition in glucose and cationic amino acid metabolism (Figs. 1 and 2). At hatch, GLUT expression was limited to the heart and liver, while CAT isoforms were expressed in most tissues. This tissue distribution may be related to glucose and amino acid metabolism occurring within these tissues just prior to hatch (Romanoff, 1967; Raheja et al., 1971). In most tissues, GLUT and CAT isoforms expressed on day 1 posthatch declined during the first week posthatch, while novel tissue GLUT and CAT isoforms were measurable on day 7. Most tissue isoforms expressed on day 1 posthatch had higher expression levels on day 14 posthatch. The decline in isoform expression between days 1 and 3 posthatch for two different nutrient transport systems suggests that a common mechanism is regulating their expression following hatch. This mechanism may be related to dietary adaptations posthatch and/or factors related to the events of hatching.

Chicks are highly adapted to protein and lipid metabolism at hatch due to utilization of these substrates throughout embryogenesis (Vince, 1974). Yolk lipid is the primary energy substrate during embryogenesis (Romanoff, 1960) and dietary glucose is not the primary energy source until yolk sac lipid stores are exhausted several days after hatching (Sklan, 2003). Intestinal absorption of dietary glucose at hatch is low (Noy and Sklan, 1999) and secretion of yolk sac lipid into the intestinal lumen for several days posthatch creates a hydrophobic environment that is unfavorable for glucose absorption (Noy and Sklan, 2001). Glucose absorption increases after hatch and glucose becomes the primary energy substrate on day 7 (Noy and Sklan, 1999, 2001; Sklan, 2003). The expression of at least one GLUT isoform in most tissues on day 7 posthatch corresponds to glucose serving as the primary energy substrate. This suggests that nutritional and/or hormonal factors are responsible for coordinating glucose transport with glucose oxidation.

The progressive decline in albumen proteins containing high lysine levels during embryogenesis (Romanoff, 1967) and the ability of lysine supplementation to increase embryo growth (Ohta et al., 2001) suggest that this amino acid is nutritionally limiting to the late stage embryo. The drop in lysine levels in ovo as chicks approach hatch may be one factor contributing to the decrease in CAT mRNA expression posthatch. Mammalian CAT-1 mRNA stability and translation is regulated equally by deprivation of a single essential amino acid or by deprivation of total amino acids (Fernandez et al., 2002). Lysine deprivation induces expression of the CHOP transcription factor (Jousse et al., 2000), indicating a potential mechanism for this amino acid to regulate CAT gene expression. CHOP is also regulated by the acute phase response (Sylvester et al., 1994). Corticosterone levels are elevated during the acute phase response (Klasing et al., 1987), and the high corticosterone levels in chicks at hatch suggest that hatching may induce an acute phase response (Latour et al., 1995). Therefore, the change in CAT expression during the 3 days following hatch may be related to factors associated with either diet or stress, or their interaction.

The ontogeny of GLUT and CAT mRNA expression in primary and secondary lymphoid tissues differed. Lymphocyte development in the bursa and thymus is initiated during embryogenesis and continues in both tissues for several weeks posthatch (Eerola et al., 1987). Bursal CAT isoforms were expressed on day 1 posthatch and GLUT isoforms were expressed on day 7 posthatch. In contrast, thymic CAT and GLUT isoforms were not expressed until day 7 and day 14, respectively. These differences indicate that the bursa may be better equipped to obtain glucose and cationic amino acids during the first week following hatch than the thymus, and that GLUT and CAT expression in these primary immune tissues is differentially regulated during the first week posthatch.

In contrast to primary immune tissues, the spleen is an immature organ at hatch, and the majority of the development of the splenic lymphoid structure and their colonization by B and T lymphocytes occurs posthatch (Sharma, 1998). Thus, the functional development of the spleen as a secondary lymphoid tissue is delayed several weeks posthatch (Eerola et al., 1987). Therefore, the low GLUT and CAT expression in the spleen following hatch may be due to the immaturity of this organ at this age.

4.2. Relative amount of each GLUT and CAT isoform mRNA within each tissue

The amount and types of GLUT isoforms expressed in tissues differed on days 7 and 14 posthatch (Tables 3 and 4). GLUT-2 was the predominant GLUT isoform expressed in the liver and no other tissue possessed this isoform. Similarly, mammalian GLUT-2 is expressed in the liver, though it is also found in intestine, kidney and pancreatic β-cells (Nordlie et al., 1999). Chicken jejunum contains a GLUT-2 type carrier in the basolateral membrane, with a Michaelis constant similar to mammalian GLUT-2 (K_m=17 mM) (Garriga et al., 1997). The Michaelis constant of GLUT-2 and insensitivity to *trans*-stimulation allows GLUT-2 to facilitate glucose export during hepatic gluconeogenesis and glucose import during hepatic glycogenesis (Thorens, 1996). Therefore, the transport characteristics of GLUT-2 control fundamental aspects of hepatic glucose metabolism in chicks.

The predominant CAT isoform in each tissue did not change between day 7 and day 14 posthatch. CAT-2 was the only isoform expressed in all tissues on days 7 and 14 posthatch, and was the predominant isoform expressed in the gastrocnemius, pectoralis and liver on both days. The mammalian CAT-2 primary transcript is differentially spliced to produce two isoforms, CAT-2A and CAT-2B (Finley et al., 1995). In our studies, total CAT-2 expression was measured since neither the full-length sequence nor splice variants of chicken CAT-2 have been identified. Chicken total CAT-2 expression was highest in the liver and skeletal muscle, similar to total CAT-2 expression patterns in human tissues (Closs et al., 1997). In mice, the liver is the only tissue expressing only one splice variant (Kakuda et al., 1998). The expression of both isoforms in most mammalian tissues warrants determination of CAT-2 splice variants in chickens. Determining the isoform composition of total CAT-2 tissue expression in chickens has implications on tissue priority since CAT-2 isoforms have contrasting transport properties.

High-affinity GLUT and CAT isoforms provide a basal level of glucose and cationic amino acid uptake, respectively. Both high affinity GLUT isoforms were expressed in all chicken tissues on day 14 posthatch. This pattern differs from mammals where GLUT-1 expression is ubiquitous and GLUT-3 expression is limited to the brain and testis (Uldry and Thorens, in press). The differing

functional role of GLUT-1 and GLUT-3 in chickens relative to mammals (Wagstaff et al., 1995) may explain this difference in tissue expression patterns. A similar difference between chickens and mammals exists for the high affinity CAT transporters. Both high-affinity CAT isoforms were expressed in most chicken tissues. However, CAT-1 is ubiquitously expressed but CAT-3 expression is limited to the brain in rodents (Ito and Groudine, 1997; Hosokawa et al., 1999).

4.3. Relative amount of GLUT and CAT isoform mRNA across tissues

The ability of tissues to acquire glucose and cationic amino acids is dependent upon qualitative and quantitative differences in GLUT and CAT expression since their transport rates are dependent upon substrate concentrations in the plasma. In chickens, plasma glucose levels are approximately 12.5 mM (Bell, 1971), and plasma lysine and arginine concentrations range from 200 to 400 μM (Klasing and Austic, 1984; Kidd et al., 2001). Assuming similar transport kinetics between chicken and mammalian GLUT and CAT systems (White et al., 1991; Wagstaff et al., 1995; Garriga et al., 1997; Torras-Llort et al., 1998), the K_m for the high-affinity GLUT and CAT isoforms is similar to or below their substrate concentrations in plasma. Thus, high-affinity GLUT and CAT isoforms are transporting substrate at rates near maximum velocity (V_{max}). The heart and bursa had the highest levels of total high-affinity GLUT and CAT mRNA expression, respectively (Table 6). If protein expression follows this pattern, the heart and bursa would have a high priority for glucose and cationic amino acids, respectively, since a greater proportion of their transporters are functioning near V_{max}. These high-priority tissues are least susceptible to fluctuations in substrate levels due to their high-affinity transporters. Skeletal muscle and liver appeared to have a low priority for glucose and cationic amino acids because they have low levels of mRNA expression for the high-affinity isoforms; however, these tissues would have high rates of substrate flux due to their high expression of low-affinity, high-capacity transporters. It would be interesting to quantify functional GLUT and CAT protein and determine glucose and lysine and arginine transport kinetics across tissues given the potential functional consequences of altered tissue GLUT and CAT mRNA levels.

The expression level of high- and low-affinity nutrient transporters by different tissues of the immune system may determine their susceptibility to fluctuations in the availability of the nutrients. Both total and high affinity CAT expression in the bursa was almost fourfold greater than in the thymus. B lymphocytes undergo important developmental and maturational events in the bursa and this is especially intense at the time of hatch and for the next several weeks, as indicated by the permanent impairment in humoral immunity caused by bursectomy at this time (Cooper et al., 1969). The short window of time around hatching during which B-cell development occurs makes it crucial for an uninterrupted nutrient supply during this period and the high expression of CATs apparently facilitates this need. On the other hand, the period of T lymphocyte development in the thymus is spread out over a much longer period of time and T-cells that migrate from the thymus prior to hatch have sufficient diversity and replication capacity to sustain cell-mediated functions (Chen et al., 1989). The low levels of GLUT and CAT isoform expression in the thymus suggests that this lymphocyte pool may be most susceptible to fluctuations in glucose and cationic amino acids.

Transitions in life stages are associated with alterations in dietary composition and represent a period of heightened metabolic adaptation. In addition, understanding nutrient acquisition by immune tissues and leukocyte populations will aid in the use of nutrition to improve animal health. The disparity in the priority of B and T lymphocyte primary immune tissues for limiting amino acids may have implications on animal health, particularly in regard to T lymphocyte homeostasis and immune responses to T-dependent antigens.

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References

Bell, D.J., 1971. Plasma glucose. In: Bell, D.J., Freeman, B.M. (Eds.), Physiology and Biochemistry of the Domestic Fowl. Academic Press, London, pp. 913–920.

Broer, S., 2002. Adaptation of plasma membrane amino acid transport mechanisms to physiological demands. Eur. J. Physiol. 444, 457–466.

Busch, W., Saier Jr., M.H., 2002. The transporter classification (TC) system, 2002. Crit. Rev. Biochem. Mol. Biol. 37, 287–337.

Carver, F.M., Shibley Jr., I.A., Pennington, J.S., Pennington, S.N., 2001. Differential expression of glucose transporters during chick embryogenesis. Cell. Mol. Life Sci. 58, 645–652.

Chen, C.H., Sowder, J.T., Lahti, J.M., Cihak, J., Losch, U., Cooper, M.D., 1989. TCR3: a third T-cell receptor in the chicken. Proc. Natl. Acad. Sci. U. S. A. 88, 2351–2355.

Chillaron, J., Roca, R., Valencia, A., Zorzano, A., Palacin, M., 2001. Heteromeric amino acid transporters: biochemistry, genetics, and physiology. Am. J. Physiol. 281, F995–F1018.

Closs, E.I., Lyons, C.R., Kelly, C., Cunningham, J.M., 1993. Characterization of the third member of the MCAT family of cationic amino acid transporters. Identification of a domain that determines the transport properties of the MCAT proteins. J. Biol. Chem. 268, 20796–20800.

Closs, E.I., Gräf, P., Habermeier, A., Cunningham, J.M., Förstermann, U., 1997. Human cationic amino acid transporters hCAT-1, hCAT-2A, and

hCAT-2B: three related carriers with distinct transport properties. Biochemistry 36, 6462–6468.
Cooper, M.D., Cain, W.A., Van Alten, P.J., Good, R.A., 1969. Development and function of the immunoglobulin producing system. Effect of bursectomy at different stages of development on germinal centers, plasma cells, immunoglobulins and antibody production. Int. Arch. Allergy 35, 242–252.
Deves, R., Boyd, C.A., 1998. Transporters for cationic amino acids in animal cells: discovery, structure, and function. Physiol. Rev. 78, 487–545.
Eerola, E., Veromaa, T., Toivanen, P., 1987. Special features in the structural organization of the avian lymphoid system. In: Toivanen, A., Toivanen, P. (Eds.), Avian Immunology: Basis and Practice. CRC Press, Boca Raton, FL, pp. 9–21.
Fernandez, J., Yaman, I., Sarnow, P., Snider, M.D., Hatzoglou, M., 2002. Regulation of internal ribosomal entry site-mediated translation by phosphorylation of the translation initiation factor eIF2alpha. J. Biol. Chem. 277, 19198–19205.
Finley, K.D., Kakuda, D.K., Barrieux, A., Kleeman, J., Huynh, P.D., MacLeod, C.L., 1995. A mammalian arginine/lysine transporter uses multiple promoters. Proc. Natl. Acad. Sci. U. S. A. 92, 9378–9382.
Garriga, C., Moreto, M., Planas, J.M., 1997. Hexose transport across the basolateral membrane of the chicken jejunum. Am. J. Physiol. 272, R1330–R1335.
Hosokawa, H., Ninomiya, H., Sawamura, T., Sugimoto, Y., Ichikawa, A., Fujiwara, K., Masaki, T., 1999. Neuron-specific expression of cationic amino acid transporter 3 in the adult rat brain. Brain Res. 838, 158–165.
Ito, K., Groudine, M., 1997. A new member of the cationic amino acid transporter family is preferentially expressed in adult mouse brain. J. Biol. Chem. 272, 26780–26786.
Joost, H.G., Bell, G.I., Best, J.D., Birnbaum, M.J., Charron, M.J., Chen, Y.T., Doege, H., James, D.E., Lodish, H.F., Moley, K.H., Moley, J.F., Mueckler, M., Rogers, S., Schurmann, A., Seino, S., Thorens, B., 2002. Nomenclature of the GLUT/SLC2A family of sugar/polyol transport facilitators. Am. J. Physiol. 282, E974–E976.
Jousse, C., Bruhat, A., Ferrara, M., Fafournoux, P., 2000. Evidence for multiple signaling pathways in the regulation of gene expression by amino acids in human cell lines. J. Nutr. 130, 1555–1560.
Kakuda, D.K., Finley, K.D., Maruyama, M., MacLeod, C.L., 1998. Stress differentially induces cationic amino acid transporter gene expression. Biochim. Biophys. Acta 1414, 75–84.
Kidd, M.T., Peebles, E.D., Whitmarsh, S.K., Yeatman, J.B., Wideman Jr., R.F., 2001. Growth and immunity of broiler chicks as affected by dietary arginine. Poult. Sci. 80, 1535–1542.
Klasing, K.C., Austic, R.E., 1984. Changes in plasma, tissue, and urinary nitrogen metabolites due to an inflammatory challenge. Proc. Soc. Exp. Biol. Med. 176, 276–284.
Klasing, K.C., Laurin, D.E., Peng, R.K., Fry, M., 1987. Immunologically mediated growth depression in chicks: influence of feed intake, corticosterone and interleukin-1. J. Nutr. 117, 1629–1637.
Latour, M.A., Peebles, E.D., Boyle, C.R., Brake, J.D., Kellogg, T.F., 1995. Changes in serum lipid, lipoprotein and corticosterone concentrations during neonatal chick development. Biol. Neonate 67, 381–386.
Liu, W., Saint, D.A., 2002. A new quantitative method of real time reverse transcription polymerase chain reaction assay based on simulation of polymerase chain reaction kinetics. Anal. Biochem. 302, 52–59.
Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25, 402–408.
MacLeod, C.L., Kakuda, D.K., 1996. Regulation of CAT: cationic amino acid transporter gene expression. Amino Acids 11, 171–191.
Mueckler, M., 1994. Facilitative glucose transporters. Eur. J. Biochem. 219, 713–725.
Nordlie, R.C., Foster, J.D., Lange, A.J., 1999. Regulation of glucose production by the liver. Annu. Rev. Nutr. 19, 379–406.
Noy, Y., Sklan, D., 1999. Energy utilization in newly hatched chicks. Poult. Sci. 78, 1750–1756.
Noy, Y., Sklan, D., 2001. Yolk and exogenous feed utilization in the posthatch chick. Poult. Sci. 80, 1490–1495.
NRC, 1994. Nutrient Requirements of Poultry. National Academy Press, Washington, DC.
Ohta, Y., Kidd, M.T., Ishibashi, T., 2001. Embryo growth and amino acid concentration profiles of broiler breeder eggs, embryos, and chicks after in ovo administration of amino acids. Poult. Sci. 80, 1430–1436.
Palacin, M., Estevez, R., Bertran, J., Zorzano, A., 1998. Molecular biology of mammalian plasma membrane amino acid transporters. Physiol. Rev. 78, 969–1038.
Paulsen, I.T., Sliwinski, M.K., Saier Jr., M.H., 1998. Microbial genome analyses: global comparisons of transport capabilities based on phylogenies, bioenergetics and substrate specificities. J. Mol. Biol. 277, 573–592.
Raheja, K.L., Snedecor, J.G., Freedland, R.A., 1971. Activities of some enzymes involved in lipogenesis, gluconeogenesis, glycolysis and glycogen metabolism in chicks (*Gallus domesticus*) from day of hatch to adulthood. Comp. Biochem. Physiol. B39, 237–246.
Ramakers, C., Ruijter, J.M., Deprez, R.H., Moorman, A.F., 2003. Assumption-free analysis of quantitative real-time polymerase chain reaction (PCR) data. Neurosci. Lett. 339, 62–66.
Romanoff, A., 1960. The Avian Embryo. The Macmillan, New York.
Romanoff, A., 1967. Biochemistry of the Avian Embryo. John Wiley & Sons, New York.
Saier, M.H., 1999. Genome archeology leading to the characterization and classification of transport proteins. Curr. Opin. Microbiol. 2, 555–561.
Sharma, J.M., 1998. Avian immunology. In: Pastoret, P., Griebel, P., Bazin, H., Govaerts, A. (Eds.), Handbook of Vertebrate Immunology. Academic Press, San Diego, pp. 73–136.
Sklan, D., 2003. Fat and carbohydrate use in posthatch chicks. Poult. Sci. 82, 117–122.
Sloan, J.L., Mager, S., 1999. Cloning and functional expression of a human Na(+) and Cl(−)-dependent neutral and cationic amino acid transporter B(0+). J. Biol. Chem. 274, 23740–23745.
Stein, W.D., 1990. Channels, Carriers, and Pumps: An Introduction to Membrane Transport. Academic Press, San Diego, CA.
Sylvester, S.L., ap Rhys, C.M., Luethy-Martindale, J.D., Holbrook, N.J., 1994. Induction of GADD153, a CCAAT/enhancer-binding protein (C/EBP)-related gene, during the acute phase response in rats. Evidence for the involvement of C/EBPs in regulating its expression. J. Biol. Chem. .269, 20119–20125.
Thorens, B., 1996. Glucose transporters in the regulation of intestinal, renal, and liver glucose fluxes. Am. J. Physiol. 270, G541–G553.
Torras-Llort, M., Soriano-Garcia, J.F., Ferrer, R., Moreto, M., 1998. Effect of a lysine-enriched diet on L-lysine transport by the brush-border membrane of the chicken jejunum. Am. J. Physiol. 274, R69–R75.
Uldry, M., Thorens, B., 2004. The SLC2 family of facilitated hexose and polyol transporters. Pflugers Arch. (in press).
Vandesompele, J., De Paepe, A., Speleman, F., 2002. Elimination of primer-dimer artifacts and genomic coamplification using a two-step SYBR green I real-time RT-PCR. Anal. Biochem. 303, 95–98.
Vince, M.A., 1974. Nutrition and utilization of albumen and yolk. In: Freeman, B.M., Vince, M.A. (Eds.), Development of the Avian Embryo. Chapman and Hall, London, pp. 160–185.
Wagstaff, P., Kang, H.Y., Mylott, D., Robbins, P.J., White, M.K., 1995. Characterization of the avian GLUT1 glucose transporter: differential regulation of GLUT1 and GLUT3 in chicken embryo fibroblasts. Mol. Biol. Cell 6, 1575–1589.
Wang, M.-Y., Tsai, M., Wang, C., 1994. Identification of chicken liver glucose transporter. Arch. Biochem. Biophys. 310, 172–179.
White, M.K., Weber, M.J., 1988. Transformation by the src oncogene alters glucose transport into rat and chicken cells by different mechanisms. Mol. Biol. Cell 8, 138–144.

White, M.K., Rall, T.B., Weber, M.J., 1991. Differential regulation of glucose transporter isoforms by the src oncogene in chicken embryo fibroblasts. Mol. Cell. Biol. 11, 4448–4454.

White, M.K., De Vente, J.E., Robbins, P.J., Canupp, D.M., Mayo, M.W., Steelman, L.S., McCubrey, J.A., 1994. Differential regulation of glucose transporter expression in hematopoietic cells by oncogenic transformation and cytokine stimulation. Oncol. Rep. 1, 17–26.

Wood, S., Trayhurn, P., 2003. Glucose transporters (GLUT and SGLT): expanded families of sugar transport proteins. Br. J. Nutr. 89, 3–9.

Yan, Q., 2003. Membrane Transporters: Methods and Protocols. Humana Press, Totowa, NJ.